#14 Declaration 2/14/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of
HUANG et al.

Group Art Unit: 1651

Application No.: 08/882,499

Examiner: Marx

Filed: June 25, 1997

For: COMPOUNDS FOR THE SUPPRESSION . . .

* * * * *

DECLARATION UNDER RULE 131

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, Ru-chih Huang, do hereby declare and state:

1. I am an inventor on Patent Application No. 08/882,499, and I am familiar with the specification and prosecution history, including the Office Action issued September 24, 1999.

2. In the September 24, 1999 Office Action, the Examiner rejected pending claims 5-7 as being anticipated by U.S. Patent No. 5,837,252 issued to Sinnott et al. on November 17, 1998. The Sinnott et al. patent was filed on October 7, 1996.

3. The invention described and claimed in Application No. 08/882,499 was made prior to October 7, 1996, as evidenced by the attached pages of laboratory notebooks dated prior to October 7, 1996. The notebook pages were recorded in Chinese. A typed English translation is attached as Tables 1 and 2.

4. The data in Table 1 shows inhibition of Herpes Simplex Virus 1 ("HSV-1") by M_4N ("4-N"). For example, at concentrations of 7.8 μg/ml or lower, while HSV-1 production

was found to be 60% or more inhibited by the standard cytopathic effect (CPE) reduction assay, there was no inhibition of cell growth at these drug concentrations (comparing column 4 to column 7). The 50% cell toxicity by M_4N (4-N) was found to be 83 μg/ml while the 50% inhibition of HSV-1 by M_4N (4-N) was found to be less than 0.98 μg/ml (comparing column 5 to column 8). This value of IC_{50} for M_4N (4-N) was comparable to that of acyclovir (ACV), a drug which is commonly used against herpes simplex viruses (Table 2). Thus, the data show suppression of viral growth by M_4N.

5. This work was carried out entirely under my direction and supervision by Dr. Hong Shan Chen and has since been published as part of a larger study (Chen et al., 1998, *J. Med. Chem.* **41**:3001-3007).

6. I declare further that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements ane the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the instant patent specification or any patent issuing thereon.

By Richard C. Huang Date Feb. 3 2000

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4N 对 Vero细胞毒性 96. 6. 25

药量 μg/ml	细胞OD值(570)	破坏%	TC50
1000	0.03 0.03 0.03 0.02	97.05	83 μg/ml
X±SD	0.03±0.01		
500	0.03 0.04 0.05 0.04	96.08	
	0.04±0.01		
250	0.3 0.3 0.3 0.3	70.59	
	0.3±0		
125	0.34 0.38 0.36 0.39	63.73	
	0.37±0.02		
62.5	0.45 0.46 0.45 0.45	55.88	
	0.45±0.01		
31.3	0.81 0.80 0.86 0.85	18.63	
	0.83±0.03		
15.6	1.08 1.02 1.00 0.98	0	
	1.02±0.04		
7.8	1.25 1.04 1.00 1.11	−0.08	
	1.1±0.11		
3.9	1.10 1.08 1.06 1.08	−0.06	
	1.08±0.02		
1.95	1.10 1.20 1.22 1.00	−0.08	
	1.1±0.1		
0.98	1.30 1.25 1.15 1.15	−0.18	
L605	1.2±0.08		

CC 1.02 1.03 1.00

1.02±0.02

VC 0 0 0.0

(2)

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4N HSV-I 抑制实验 96.6.25

药量 μg/ml	O.D值 (570 nm)	抑制率 %	IC50 (μg/ml)
1000	0.04 0.01 0.00 0.00	92.86	< 0.98
	0.02±0.02		
500	0.05 0.11 0.07 0.04	72.50	
	0.07±0.03		
250	0.30 0.26 0.12 0.18	41.7	
	0.27±0.03		
125	0.57 0.45 0.62 0.60	31.5	
	0.56±0.08		
62.5	0.52 0.49 0.68 0.66	34.8	
	0.59±0.10		
31.3	0.90 0.84 0.79 0.87	64.00	
	0.85±0.05		
15.6	0.75 0.92 0.68 0.92	59.6	
	0.81±0.13		
7.8	0.74 0.79 0.96 1.03	67.4	
	0.88±0.14		
3.9	0.79 1.07 0.83 0.76	65.2	
	0.86±0.14		
1.95	0.78 0.85 0.81 0.76	78.4	
	0.80±0.04		
0.98	0.83 0.73 0.80 0.83	84.4	
	0.80±0.05		
CC	1.13 1.22 1.18 1.15		
	1.17±0.04		
VQ L605	0.24 0.29 0.31 0.26		
	0.28±0.03		

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ACN 对HSV-I 作用 96.6.25

浓度 μg/ml	OD值	抑制率%	IC50 μg/ml
1000	0.70 0.53 0.57 0.62	37	<0.98
	0.61±0.07		
500	0.63 0.38 0.62 0.49	28.1	
	0.53±0.12		
250	0.50 0.49 0.64 0.87	39.3	
	0.63±0.18		
125	0.41 0.43 0.53 0.66	45.8	
	0.51±0.11		
62.5	0.73 0.73 0.73 0.76	51.7	
	0.74±0.02		
31.3	0.93 0.90 0.86 0.93	70.8	
	0.91±0.03		
15.6	1.00 0.99 0.98 1.02	80.9	
	1.00±0.02		
7.8	1.09 1.11 1.16 1.10	94.4	
	1.12±0.03		
3.9	1.13 1.07 1.10 1.09	92.1	
	1.10±0.02		
1.95	1.14 1.12 1.17 1.11	96.6	
	1.14±0.03		
0.98	0.76 0.80 0.82 0.92	61.8	
	0.83±0.07		
SC	1.13 1.22 1.18 1.15		
	1.17±0.04		

VCL605 0.24 0.29 0.31 0.26
0.28±0.03

实验者：陈鸿珊 娜53
1991 6.27

L996,6,25,給葛爾法官

C-4N Doc

Table 1. Cytotoxicity and inhibition of HSV-1 CPE by 4N in vero cell cultures

1996, 6-20-25

Agent	Conc. ug/ml	Cytotoxicity(OD570) X ± SD	Inhibition of cell growth %	TC50 ug/ml	HSV-1 CPE (OD570) X ± SD	Inhibition of HSV-1CPE %	IC50 ug/ml
4-N	1000.00	0.03, 0.03, 0.03, 0.02 0.03 ± 0.01	97.05	83	0.04, 0.05, 0.00, 0.00 0.02± 0.03	-	<0.98
	500.00	0.03, 0.04, 0.05, 0.04 0.04± 0.01	96.08		0.05, 0.11, 0.07, 0.04 0.07± 0.03	-	
	250.00	0.30, 0.30, 0.30, 0.30 0.3± 0	70.59		0.30, 0.26, 0.22, 0.28 0.27± 0.03	-	
	125.00	0.34, 0.38, 036, 0.39 0.37± 0.02	63.73		0.57, 0.45, 0.62, 0.60 0.56± 0.08	31.5	
	62.50	0.45, 0.46, 0.45, 0.45 0.45± 0.01	55.88		0.52, 0.49, 0.68, 0.66 0.59± 0.10	34.8	
	31.30	0.81, 0.80, 0.86, 0.85 0.83± 0.03	18.63		0.90, 0.84, 0.79, 0.87 0.85± 0.05	64	
	15.60	1.08, 1.02, 1.00, 0.98 1.02± 0.04	0		0.72, 0.92, 0.68, 0.92 0.81± 0.13	59.6	
	7.80	1.25, 1.04, 1.00, 1.11 1.10± 0.11	-0.08		0.74, 0.79, 0.96, 1.03 0.88± 0.14	67.4	
	3.90	1.10, 1.08, 1.06, 1.08 1.08± 0.02	-0.06		0.79, 1.07, 0.83, 0.76 0.86± 0.14	65.2	
	1.95	1.10, 1.20, 1.22, 1.00 1.10± 0.1	-0.08		0.78, 0.85, 0.81, 0.76 0.80± 0.04	58.4	
	0.98	1.30, 1.25, 1.15, 1.15 1.20± 0.08	-0.18		0.83, 0.73, 0.80, 0.83 0.80± 0.05	58.4	
	CC	1.02, 1.03, 1.00, 1.04 1.02± 0.02			1.13, 1.22, 1.18, 1.15 1.17± 0.04		
	vc X± SD				0.24, 0.29, 0.31, 0.26 0.28± 0.03		

Table 2. Inhibition of HSV-1 CPE by ACV on vero cell

1996, 6, 20-25.

Agent	conc. ug/ml	HSV-1 CPE(OD570)	Inhibition %	IC50 ug/ml
ACV	1000 X± SD	0.70,0.53,0.57,0.62 0.61± 0.07	37	<0.98
	500 X± SD	0.63,0.38,0.62,0.49 0.53± 0.12	28.1	
	250 X± SD	0.50,0.49,0.64,0.87 0.63± 0.18	39.3	
	125 X± SD	0.41,0.43,0.53,0.66 0.51± 0.11	25.8	
	62.5 X± SD	0.73,0.72,0.73,0.76 0.74± 0.02	51.7	
	31.3 X± SD	0.93,0.90,0.86,0.93 0.91± 0.03	70.8	
	15.6 X± SD	1.00,0.99,0.98,1.02 1.00± 0.02	80.9	
	7.8 X± SD	1.09,1.11,1.16,1.10 1.12± 0.03	94.4	
	3.9 X± SD	1.13,1.07,1.10,1.09 1.10± 0.03	92.1	
	1.95 X± SD	1.14,1.12,1.17,1.11 1.14± 0.03	96.6	
	0.98 X± SD	0.76,0.80,0.82,0.92 0.83± 0.07	61.8	
	CC X± SD	1.13,1.22,1.18,1.15 1.17± 0.04		
	VC X± SD	0.24,0.29,0.31,0.26 0.28± 0.03		